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1 MALWMRLLPL LALLALWGPD PAAAFVNGHL CGSHLVEALY LVCGERGFFY
51 TPKTRREAED LQVGQVELGG GPGAGSLQPL ALEGLGKRG IVEQCCTSIC
101 SLYQLENYCN

FIG._1A

A-chain	B-chain
----- -----	
1 GIVEQCCTSI CSLYQLENYC NFNQHLCGS HLVEALYLCV GERGFFYTPK T	

FIG._1B**1TRZ:A**

1 GIVEQCCTSI CSLYQLENYC N
HHHHTTS HHHHGGG

1TRZ:B

1 FVNQHLCGSH LVEALYLVCG ERGFFYTPKT
S HHH HHHHHHHHHG GG

1TRZ:C

1 GIVEQCCTSI CSLYQLENYC N
HHHHSSS HHHHGGG

1TRZ:D

1 FVNQHLCGSH LVEALYLVCG ERGFFYTPKT
HHHHHHH HHHHHHHHHG GG S

FIG._1C

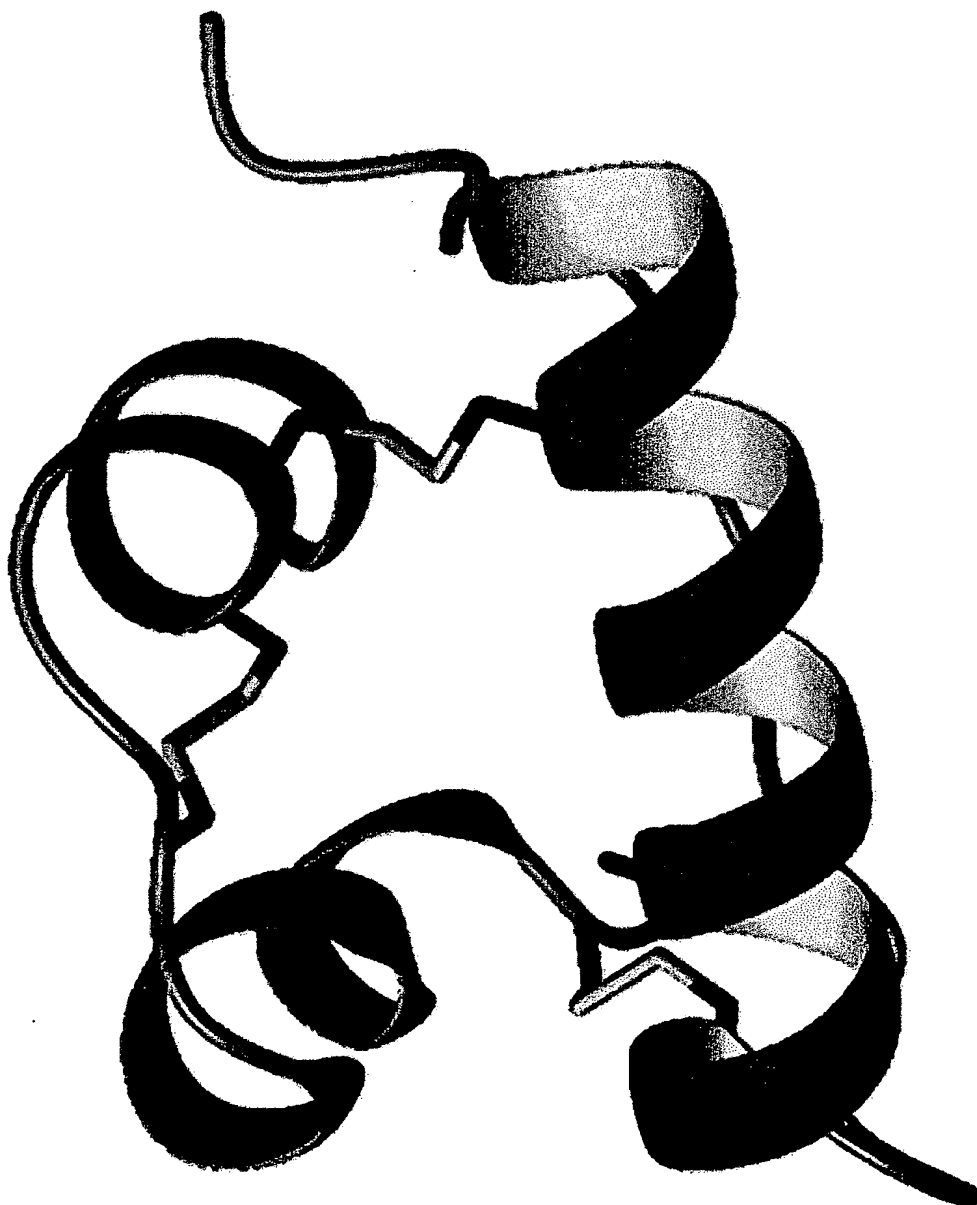


FIG.-2A

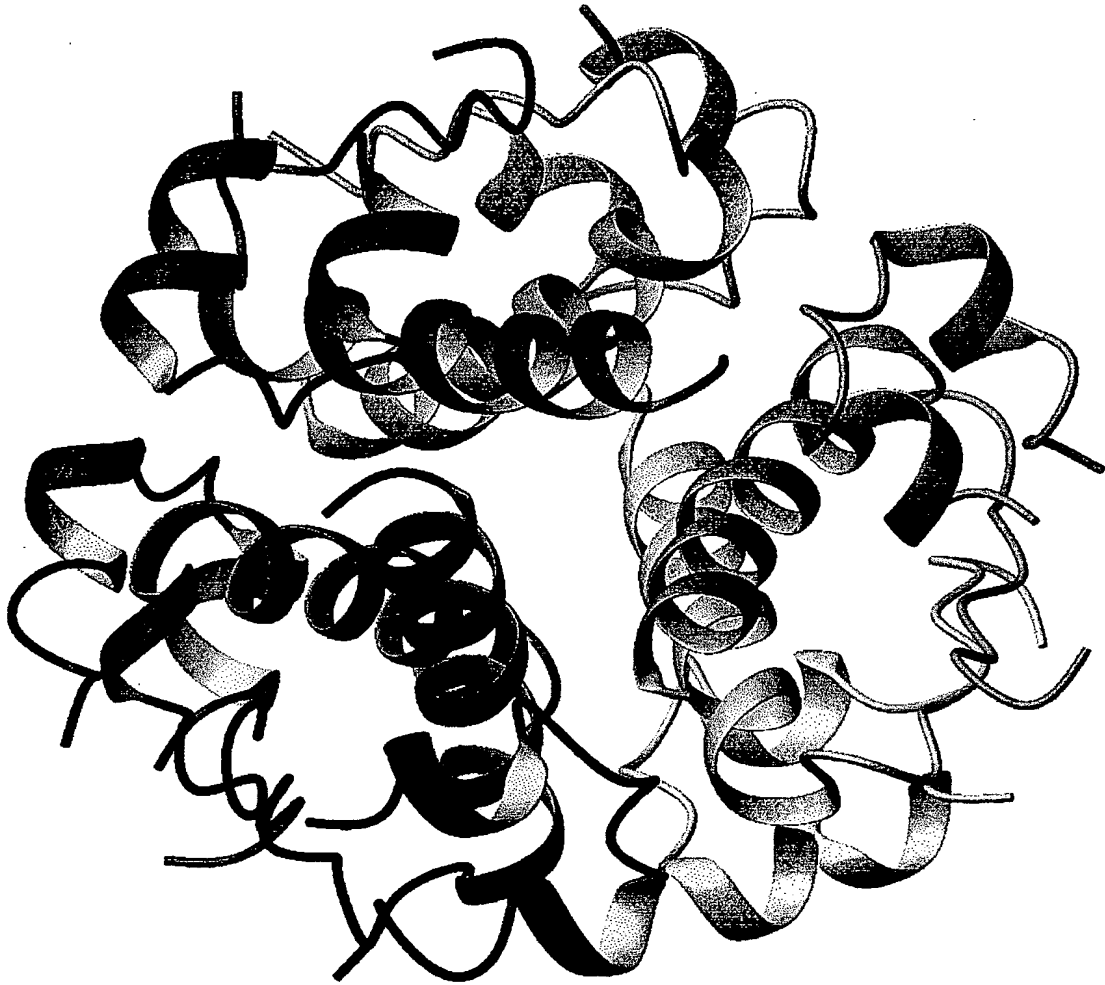


FIG. 2B

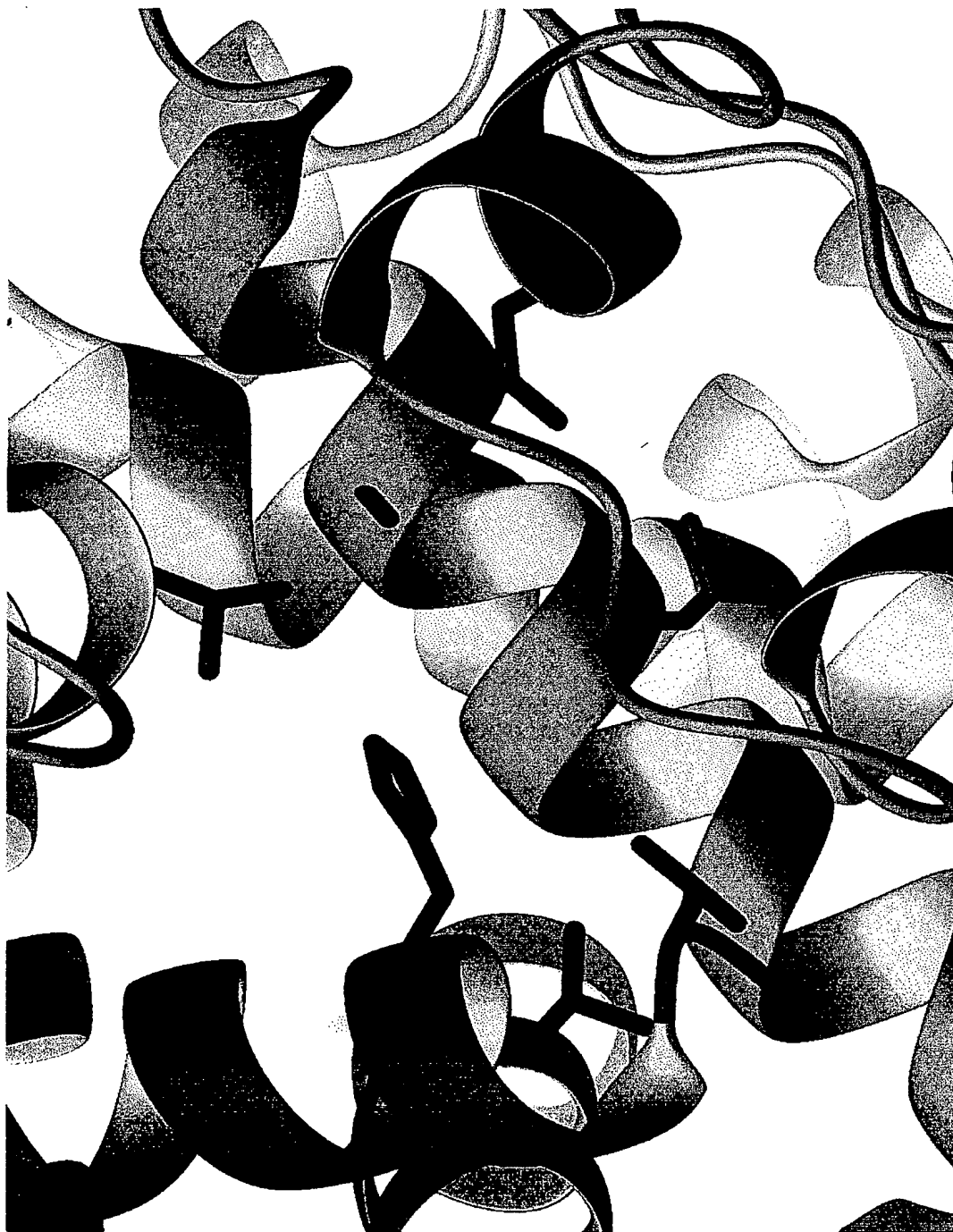
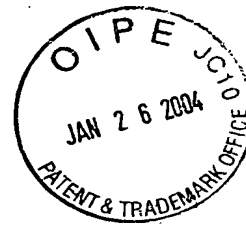
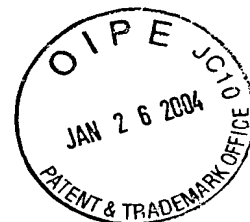


FIG. 2C





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A-chain

B-chain

1 NLVEQASTSQ ASLYQIYNFD NDVNFHLYGS HIREWLYLVA GERGFNFDPK T

FIG._3A

A-chain

B-chain

1 GIVEQCSTSI CSLYQLENYC NFENYHLYGS HLVEALYLVC GERGFFYTPK T

FIG._3B

A-chain

B-chain

1 GIVEQCSTSI CSLYQLENYC NFVNQHLDGS HLVEALYLVC GERGFFYTPK T

FIG._3C

A-chain

B-chain

1 GIVEQCSTSI CSLYQLENYC NFTNYHLYGS HLVEALYLVC GERGFFYTPK T

FIG._3D

A-chain

B-chain

1 GIVEQCSTSI CSLYQLENYC NFVNYHLYGS HLVEALYLVC GERGFFYTPK T

FIG._3E

A-chain

B-chain

1 GIVEQCSTSI CSLYQLENYC NFVNQHLEGS HLVEALYLVC GERGFFYTPK T

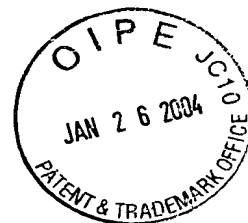
FIG._3F

A-chain

B-chain

1 GIVEQCETSI CSLYQLENYC N----HLEGS HLVEALYLVC GERGFFYTPK T

FIG._3G



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A-chain

B-chain

1 GIVEQCCTSI CSLYQLENYC NFVNQFLCGS HLVEFLYLVLC GERGFFYTPK T**FIG._4A**

A-chain

B-chain

1 GIVEQCCTSI CSLYQLENYC NFVNQFLCGS HLVEALLYLVLC GERGFFYTPK T**FIG._4B**

A-chain

B-chain

1 GIVEQCCTSI CSLYQLENYC NFVNQHLCGS HLVEFLYLVLC GERGFFYTPK T**FIG._4C**

A-chain

B-chain

1 GIVEQCCTSI CSLYQLENYC NFVNQHLCGS HLVEWLYLVLC GERGFFYTPK T**FIG._4D**

A-chain

B-chain

1 GIVEQCCTSI CSLYQLENYC NFVNQFLCGS HLVEWLYLVLC GERGFFYTPK T**FIG._4E**

A-chain

B-chain

1 GIVEQCCTSI CSLYQLENYC NFVNQHLCGS HLVEYLYLVLC GERGFFYTPK T**FIG._4F**

A-chain

B-chain

1 GIVEQCCTSI CSLYQLENYC NFVNQHLCGS HLVEILYLVLC GERGFFYTPK T**FIG._4G**



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A-chain

B-chain

1 NIVEQCCTSQ CSLYQYENYC NDVNQHLCGS HLVEALYLVC GERGFNYDPK T

FIG._5A

A-chain

B-chain

1 NIVEQCCTSQ CSLYQYYNFC NDKNFHLCGS HIREWLYLVC GERGFNFDPK T

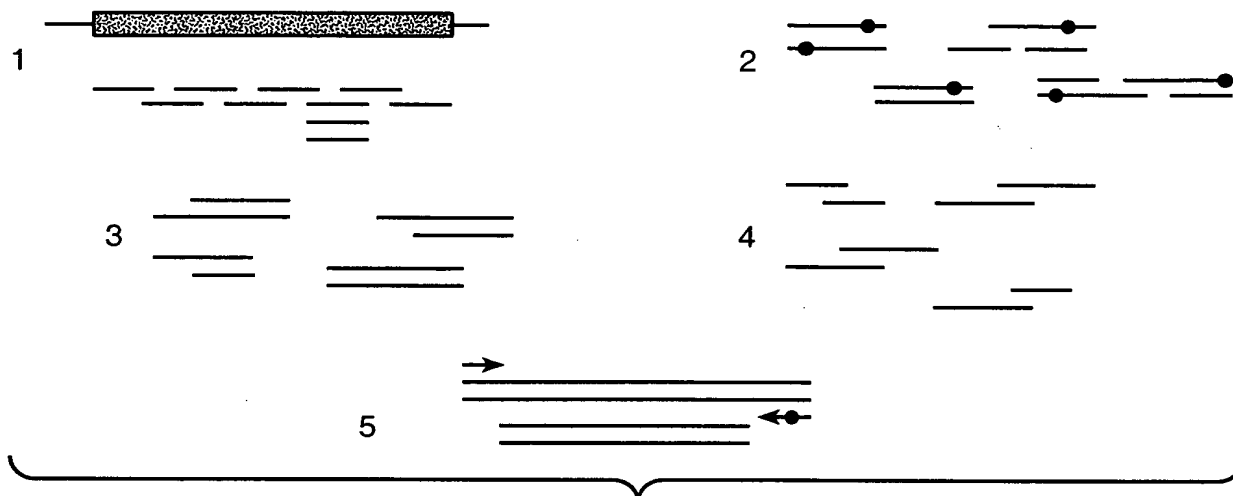
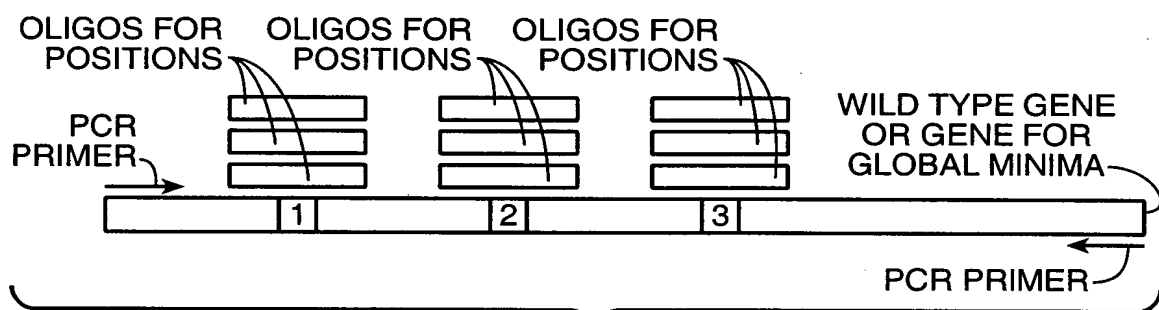
FIG._5B

A-chain

B-chain

1 NIVEQCCTSQ CSLYQYYNFC NDKNFHLCLS HIREWLYLVC GERGFNFDNK T

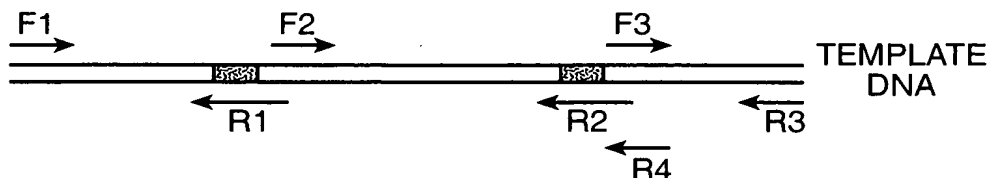
FIG._5C

**FIG._6****FIG._7**



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BLACK BOX =
REGION TO
BE MUTATED



STEP 1: SET UP 3 PCR REACTIONS:

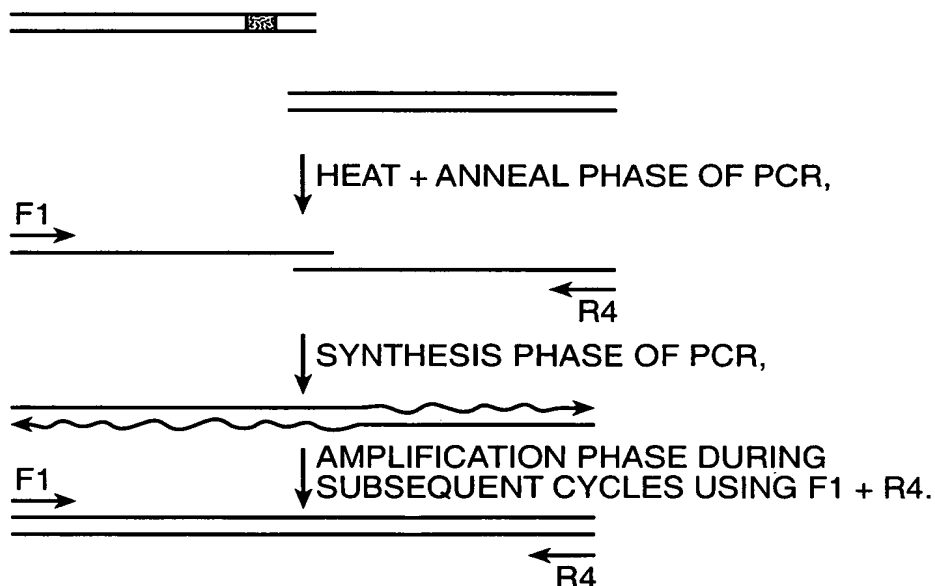
PRODUCTS:

TUBE 1:

TUBE 2:

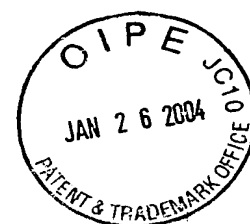
TUBE 3:

STEP 2: SET UP PCR REACTION WITH PRODUCTS OF TUBE 1 +
PRODUCTS TUBE 2 + F1 + R4.

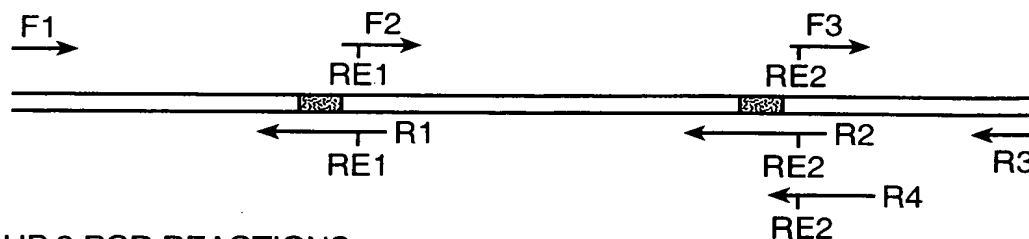


STEP 3: REPEAT STEP 2 USING PRODUCT FROM STEP 2 + PRODUCT
FROM STEP 1, TUBE 3 + PRIMERS F1 + R3.

FIG._8



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STEP 1: SET UP 3 PCR REACTIONS:

TUBE 1:

TUBE 2:

TUBE 3:

STEP 2: DIGEST PRODUCTS FROM STEP 1 WITH SUITABLE RESTRICTION ENDONUCLEASES.

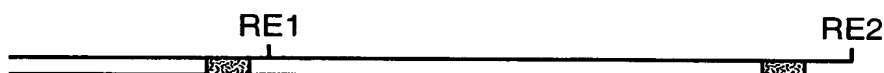
STEP 3: LIGATE DIGESTED PRODUCT FROM STEP 2, TUBE 2 WITH DIGESTED PRODUCT FROM STEP 2, TUBE 1.



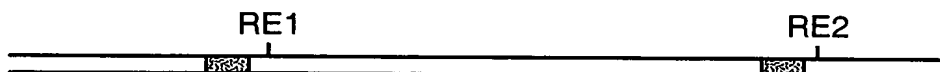
STEP 4: AMPLIFY VIA PCR LIGATED PRODUCTS OF STEP 3 WITH F1 + R4.



STEP 5: DIGEST AMPLIFIED PRODUCT OF STEP 4 WITH RESTRICTION ENDONUCLEASE #2.



STEP 6: LIGATE PRODUCT FROM STEP 5 WITH PRODUCT FROM STEP 2, TUBE 3.



STEP 7: AMPLIFY PRODUCT FROM STEP 6 WITH F1 + R3.

FIG. 9

DIAGRAM 3

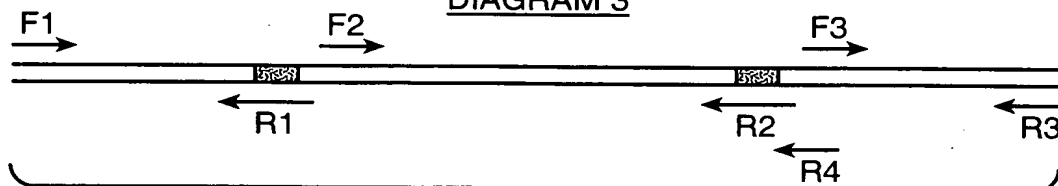


FIG. 10